



doi:10.1016/j.gca.2004.02.020

Microbial hydrocarbon gases in the Witwatersrand Basin, South Africa: Implications for the deep biosphere

J. A. WARD,¹ G. F. SLATER,² D. P. MOSER,³ L.-H. LIN,⁴ G. LACRAMPE-COULOUME,¹ A. S. BONIN,⁵ M. DAVIDSON,⁴ J. A. HALL,⁴
B. MISLOWACK,⁴ R. E. S. BELLAMY,⁶ T. C. ONSTOTT,³ and B. SHERWOOD LOLLAR^{1,*†}

¹Stable Isotope Laboratory, University of Toronto, Toronto, Ontario M5S 3B1 Canada

²Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543-1543, USA

³Environmental Microbiology Group, Pacific Northwest National Laboratory, Richland, Washington 99352, USA

⁴Department of Geosciences, Princeton University, Princeton, New Jersey 08544, USA

⁵Department of Biology, Portland State University, Portland, Oregon 97201, USA

⁶Geology Department, Beatrix Mine, Welkom 9460 South Africa

(Received July 7, 2003; accepted in revised form February 9, 2004)

Abstract—In this study, compositions and $\delta^{13}\text{C}$ and $\delta^2\text{H}$ isotopic values of hydrocarbon gases from 5 mines in the Witwatersrand basin, South Africa, support the widespread occurrence of microbially produced methane in millions of years-old fissure waters. The presence of microbial methane is, to a large extent, controlled by the geologic formations in which the gases are found. Samples from the Witwatersrand Supergroup have the largest microbial component based on $\delta^{13}\text{C}$ and $\delta^2\text{H}$ signatures and CH_4/C_2+ values. Based on mixing between a microbial CH_4 component and a more ^{13}C -enriched and ^2H -depleted C_2+ -rich end member, conservative estimates of the % contribution of microbial CH_4 to the gas samples range from >90% microbial CH_4 at Beatrix, Masimong, and Merriespruit, to between 5 and 80% microbial CH_4 at Evander, and <18% microbial CH_4 at Kloof. The Witwatersrand basin's history of thermal alteration of organic-rich ancient sedimentary units suggests a thermogenic origin for this ^{13}C -enriched end member. Alternatively, the potential for an abiogenic origin similar to hydrocarbon gases produced by water-rock interaction at other Precambrian Shield mines is discussed. Microbial methane is predominantly found in paleo-meteoric fissure waters with $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values that fall on the meteoric waterline, and have temperatures between 30 to 40°C. In contrast, fissure waters with a larger component of nonmicrobial hydrocarbon gases show a trend towards more enriched $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values that fall well above the meteoric waterline, and temperatures of 45 to 60°C. The enrichment in ^{18}O and ^2H in these samples, and their high salinity, are similar to the isotopic and compositional characteristics of saline groundwaters and brines produced by water-rock interaction at Precambrian Shield sites elsewhere. The reported 100 Ma ages of fissure waters from the Witwatersrand and Ventersdorp formations suggest that these microbial hydrocarbon gases are the product of *in situ* methanogenic communities in the deep subsurface of the Witwatersrand basin. Small subunit ribosomal RNA genes were amplified using archaeal-specific primer sets from DNA extracts derived from several of these waters. Fissure waters with a high proportion of microbial methane also contained sequences resembling those of known methanogens. Copyright © 2004 Elsevier Ltd

1. INTRODUCTION

Substantial quantities of hydrocarbon gases have been observed within Precambrian rocks of the Canadian Shield (Sherwood Lollar et al., 1993a; Sherwood Lollar et al., 1993b), the Fennoscandian Shield (Nurmi et al., 1988; Sherwood Lollar et al., 1993a), the Khibina, Lovozero, and Kovdor intrusions of the Kola Alkaline Province in Russia (Nivin et al., 1995; Potter et al., 1998; Voytov, 1991), and the Witwatersrand basin in South Africa (Cook, 1998). In general, hydrocarbon gases can be divided into two types based on origin. Biogenic hydrocarbon gas refers to gas produced directly by microorganisms during respiratory and fermentative processes (microbial gas); or, it can refer to methane and higher hydrocarbons derived from the thermal breakdown of more complex organic matter (thermogenic gas) (Schoell, 1988). Biogenic processes are the most common means of hydrocarbon gas formation, and a

substantial body of literature and experience has been established to characterize these sources based on isotopic and compositional analyses (Schoell, 1983; Schoell, 1984; Whiticar et al., 1986; Schoell, 1988; Hunt, 1996). At a number of Precambrian Shield sites, the presence of abiogenic hydrocarbon gases has been suggested (Sherwood Lollar et al., 1993a; Sherwood Lollar et al., 1993b; Nivin et al., 1995; Sherwood Lollar et al., 2002b; Potter and Konnerup-Madsen, 2003). Abiogenic synthesis of hydrocarbons involves the production of gases without the action of microorganisms or the breakdown of a high molecular weight organic precursor (Schoell, 1988). Mantle derived abiogenic gases have a CH_4 component that is significantly enriched in ^{13}C compared to microbial or thermogenic CH_4 , due to the juvenile carbon source (Welhan and Craig, 1979; Welhan, 1988; Abrajano et al., 1988). Abiogenic hydrocarbon gases can also be produced from crustal carbon sources (graphite, CO , CO_2 , or dissolved inorganic carbon) by processes such as surface-catalysed polymerization (Anderson, 1984); metamorphism of graphite-carbonate bearing rocks (Giardini and Salotti, 1969; Holloway, 1984; Kenney et al., 2002); and other gas-water-rock alteration reactions in

* Author to whom correspondence should be addressed (bslollar@chem.utoronto.ca).

† Present address: Department of Geology, University of Toronto, Toronto, Ontario M5S 3B1 Canada.

the presence of catalytically active metals, such as serpentinization (Vanko and Stakes, 1991; Charlou and Donval, 1993; Berndt et al., 1996; McCollom and Seewald, 2001; Kelley et al., 2001). Experimental results confirm that production of abiogenic CH₄ from crustal carbon sources by water-rock interactions can result in $\delta^{13}\text{C}$ values as depleted as -50‰ (Yuen et al., 1990; Hu et al., 1998; Horita and Berndt, 1999).

Within the hard rock mines of Witwatersrand basin, South Africa, hydrocarbon gases, saline groundwaters, and brines are encountered when drilling into faults and/or dyke structures (Cook, 1998). Some compositional analyses of the subsurface gases have been obtained by various mines (Cook, 1998). Few analyses, however, have been done to determine both compositions and stable isotopic signatures, which are the most reliable means of identifying the origin of hydrocarbon gases. It was first suggested that the gases within the Witwatersrand basin originated from shallow coal deposits in the basin and were transported as dissolved phases in groundwater into the underlying strata via cross-cutting igneous dykes intruded into the Witwatersrand sediments (Eschenburg, 1980; Tweedie, 1986; Robb and Meyer, 1995; Cook, 1998). Others have suggested that the gases were formed within the basin by the thermal conversion of shale layers and/or thin carbon seams (Cook, 1998). Alternatively, the gases could be similar to abiogenic gases found in other Precambrian Shield environments such as the Canadian and Fennoscandian Shields (Sherwood Lollar et al., 1993a; Sherwood Lollar et al., 1993b; Sherwood Lollar et al., 2002b).

A growing body of evidence supports the presence of indigenous microbial communities to considerable depth in the Witwatersrand basin, and thus, the possibility that at least some of the methane is microbially produced must be considered. Known microbes capable of producing methane gas are confined to only one domain of life, the Archaea (Woese and Olsen, 1986). Anaerobic microorganisms of various physiotypes have been cultivated from boreholes receiving Witwatersrand basin fissure waters (Kieft et al., 1999; Takai et al., 2001). However, to date, the report of Bonin and Boone (2004) represents the only documented cultivation of methanogens. Detailed examinations of fissure water microbial community structure have been conducted using culture-independent approaches (e.g., small subunit ribosomal RNA gene sequences (SSU rDNA) (Takai et al., 2001; Baker et al., 2003; Moser et al., 2003a; Moser et al., 2003b), or phospholipid fatty acid (Piffner et al., 2000)). Archaeal SSU rDNA sequences resembling those of methanogens have been detected in a previous survey of Witwatersrand deep mine samples, but were limited to mine service water and dolomite aquifer samples (Takai et al., 2001).

The objective of this study was to use gas compositions and stable isotope signatures to constrain the origin of hydrocarbon gases at five sites located in the Witwatersrand and Ventersdorp formations in the Witwatersrand basin, South Africa. These data were coupled with archaeal SSU rDNA sequences amplified by polymerase chain reaction (PCR) from microbial cells filtered from associated fracture waters from a number of the boreholes for which gases were sampled. The presence or absence of sequences resembling those of known methanogenic archaea was employed as a qualitative screen for methanogens in these samples. Here we report on patterns of microbially

produced hydrocarbons (or mixtures thereof) that correspond with the host rock type and support this geochemical and isotopic evidence with rDNA sequences for likely methanogens.

2. GEOLOGICAL SETTINGS AND SAMPLING LOCATIONS

The Witwatersrand basin is a large Archean intracratonic basin composed of volcanosedimentary sequences divided chronologically into the schist basement, the sedimentary quartzite and shale layers of the Witwatersrand Supergroup, and the andesitic lava sequence of the Ventersdorp Supergroup (Coward et al., 1995). The NE-SW elongated basin (Fig. 1) is approximately 360 km by 200 km. Evander Mine and Middelbult Colliery are located in the northeast portion of the basin. The Witwatersrand Supergroup (approx. 3000 to 2800 Ma) overlies the schist basement (>3070 Ma) and is the main focus for mining in the Witwatersrand basin (Fig. 2a). The Witwatersrand Supergroup has been metamorphosed to lower greenschist facies and divided into two groups based on depositional characteristics. The lower part of the Witwatersrand Supergroup (the West Rand Group) is composed of marine distal shelf facies with a minor intertidal component. They are characterized as sands, greywacke, shales, and argillites with minor-banded ironstones and can reach a maximum thickness of 7500m (Coward et al., 1995). The upper part of the Witwatersrand Supergroup (Central Rand Group) is composed of fragments derived from the eroded basement. It is characterized by an upwards-coarsening depositional pattern of fluvial sands, quartzites and conglomerates with minor shale layers that reach a maximum thickness of 2900m. Overlying the Central Rand Group is the thick sequence of andesitic lavas that comprises the Ventersdorp Supergroup (2700 Ma). This layer is comprised of up to 1840m of bimodal volcanics, thinner layers of sandstone and conglomerate, and a layer of tholeiitic flood basalt (Coward et al., 1995). The organic-rich Black Reef quartzite deposit marks the transition between the Ventersdorp Supergroup and the Transvaal Supergroup (2000–2500 Ma) (Tweedie, 1986; Coward et al., 1995). The Transvaal sediments consist of a thick dolomitic unit overlain by terrigenous sediments. Uplift of the Vredefort Dome at the centre of the Witwatersrand basin (2025 Ma) (Fig. 1) resulted in deformation of the Transvaal sediments, the Ventersdorp, and the Witwatersrand Supergroups (Coward et al., 1995). Eroded, uplifted structures gave rise to the younger (~ 200 million yr old) Karoo sedimentary basin. The Karoo sequence is composed mainly of interbedded shale and sandstone layers only a few hundred meters in thickness, with a thin layer (5m) of low-grade bituminous coal near its base (Tweedie, 1986). At Evander, samples were collected from boreholes in the underground workings in the Witwatersrand Supergroup at depths between 1474 mbls (meters below land surface) and 1950 mbls (Fig. 2a). In this area of the basin, the Transvaal has been partially removed by pre-Karoo erosion. At Middelbult, samples were collected from boreholes extending from surface to 122 to 200 mbls to the bituminous coal seam at the base of the Karoo sediments.

Kloof Mine is located in the northwest section of the Witwatersrand basin where the Karoo sediments have been removed by erosion (Fig. 2b) (Johnson et al., 1996). Gas samples

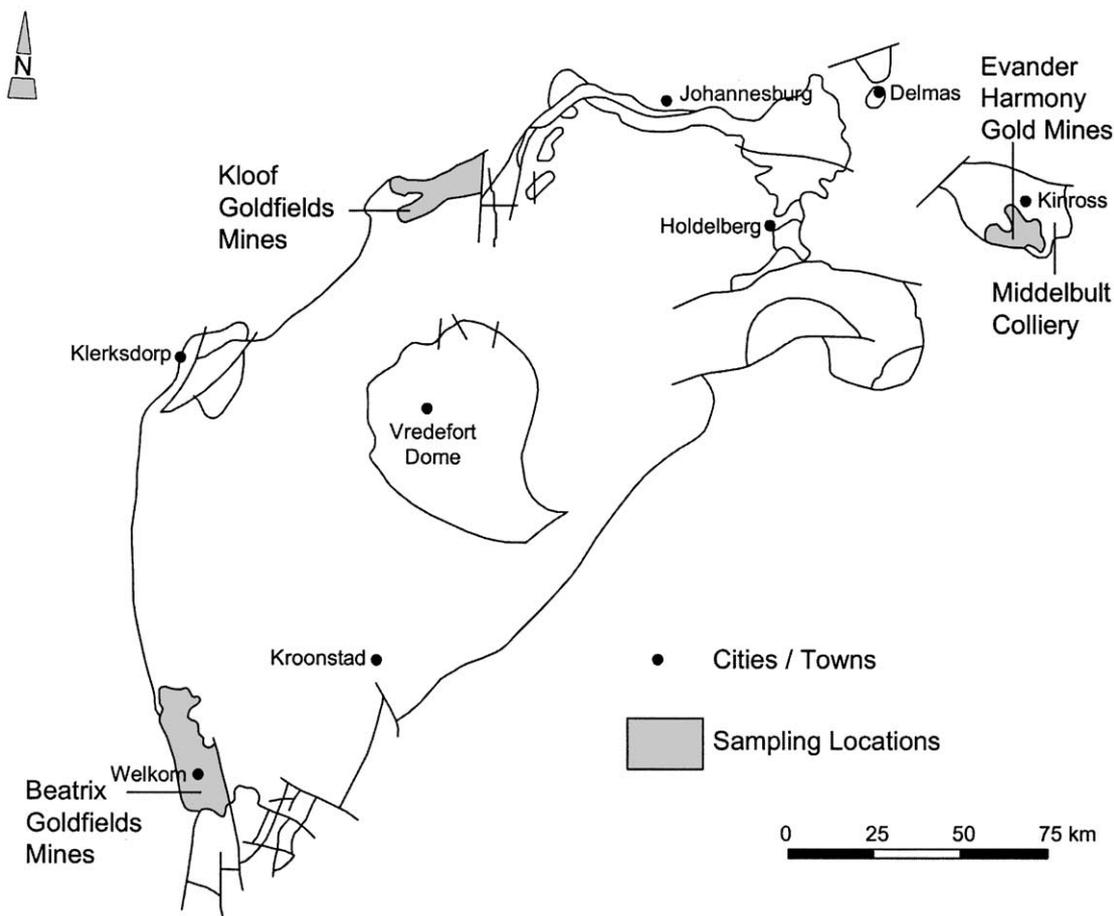


Fig. 1. Location of sampling sites within the Witwatersrand basin. Merriespruit and Masimong Mines are located within 10 km of Beatrix Mine.

at this site were exclusively from the Ventersdorp Supergroup at depths of 3300 to 3400 mbls. Beatrix, Merriespruit, and Masimong Mines are all located in the southern part of the basin near Welkom, where the Karoo sediments unconformably overlie the Ventersdorp Supergroup (Fig. 2c). All samples in these mines were collected from underground boreholes in the Central Rand Group of the Witwatersrand Supergroup. Samples at Beatrix were from 718 to 1390 mbls, while samples from Masimong and Merriespruit Mines were from 1880 and 2028 mbls respectively.

Carbonaceous matter occurs in the form of stratiform carbon seams and spherical solid bitumen nodules throughout the Witwatersrand basin (Spangenberg and Frimmel, 2001). The seams are only a few centimeters thick, and nodules of solid bitumen are up to 1 cm in diameter. They are typically found on bottom scour surfaces and wrapped around quartzite pebbles within the West Rand and Central Rand Groups (Carbon Leader/Ada May or Main Reef), within the Central Rand Group (Bird/Beatrix/Kimberly Reefs), at the base of the Ventersdorp Supergroup (Ventersdorp Contact Reef), and between the Ventersdorp Supergroup and the Transvaal sediments (Black Reef) (Figs. 2 a–c; Spangenberg and Frimmel, 2001). The source has been the subject of considerable debate (Robb and Meyer, 1995), but the general consensus is that it originated from late

Archean algal-bacterial kerogen derived from extensive microbial mats that once covered the surface of the Witwatersrand clastic sediments (Spangenberg and Frimmel, 2001).

3. MATERIAL AND METHODS

All samples were collected at the borehole collar. A packer was placed into the opening of the borehole and sealed to the inner rock walls below water level to seal the borehole from the mine air and minimize air contamination. Gas and water were allowed to flow through the apparatus long enough to displace any air remaining in the borehole or the apparatus before sampling. Plastic tubing was attached to the end of the packer, and the flow of gas and/or water from the borehole was directed into a graduated sampling bucket. If both gas and water flowed from the borehole, both flows were measured. The water flow rate was determined from the average time required for each of three fillings of a graduated sampling bucket. Gas flow was measured by filling the sampling bucket with water, then displacing a known volume of water from an inverted graduated beaker after Fritz et al. (1987). When there was no water, but flowing gas present in the borehole, the gas flow was measured in an inverted cylinder from a bucket filled with mine service water (water used in mining and drilling operations). For each borehole, gas flow rates were also averaged from three measurements. In several cases, gas and water flow rates varied significantly during sampling, so a range is reported (Table 1).

Following flow measurement, gases collected in the inverted beaker were transferred directly into preevacuated vials through a 22-g syringe needle on a luer attachment at the top of the beaker. The gas sampling

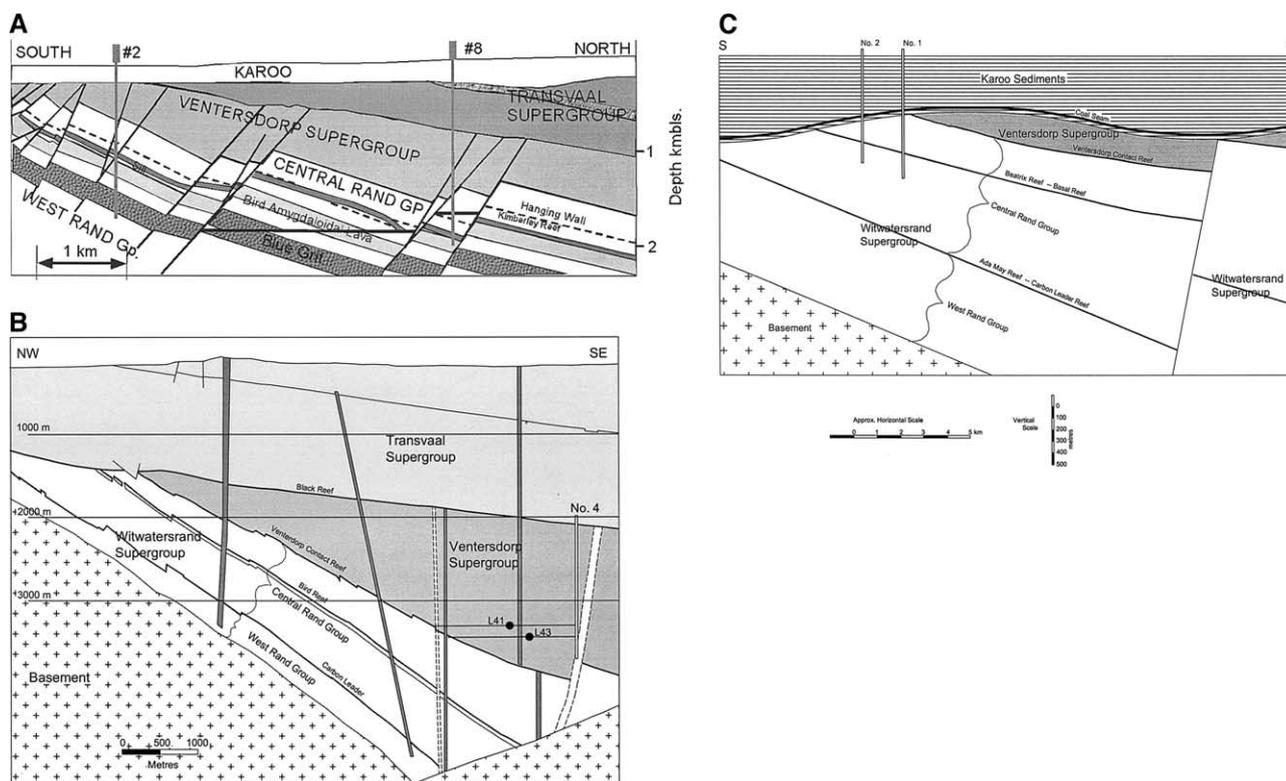


Fig. 2. Geological cross sections for sampling locations (a) Evander and Middelbult mines after Tweedie (1986); (b) Kloof mine cross section after van Heerden (1998); (c) cross section of the area of the Witwatersrand basin around Beatrix, Merriespruit, and Masimong mines after Gold Uranium and Platinum Division Geology Department of Beatrix Mines LTD.

vials were preevacuated 130 ml borosilicate vials sealed with butyl blue rubber stoppers. All stoppers were pretreated by boiling in 0.1 mol/L NaOH for an hour (Oremland and Des Marais, 1983). Vials were prefixed with 50 μ L of a saturated $HgCl_2$ solution to kill any microbes contained in the sample so microbial activity postsampling would not alter the gas composition and isotopic signatures. Six vials were filled from the gas collected in the inverted beaker and overpressurized by adding 20cc of the borehole water. All samples were stored inverted at room temperature until analysis was performed within three months following sampling. This amount of storage time has been previously shown to have no effect on the isotope signatures of the gases (Ward, 2002).

Compositional analyses of gas samples were performed at the Stable Isotope Laboratory at the University of Toronto. A Varian 3400 gas chromatograph (GC) equipped with a flame ionization detector (FID) was used to determine the concentrations of CH_4 , C_2H_6 , C_3H_8 , and C_4H_{10} . The hydrocarbons were separated on a J&W Scientific GS-Q column (30 m x 0.32 mm ID) with a helium gas flow and the following temperature program: initial 60°C hold 2.5 min, increase to 120°C at 5°C/min. A Varian 3800 GC equipped with a micro-thermal conductivity detector (μ TCD) and a Varian Molecular Sieve 5A PLOT fused silica column (25 m x 0.53 mm ID) were used to determine the concentrations of the inorganic gas components (H_2 , He, Ar, O_2 , CO_2 , and N_2). To determine the concentrations of Ar, O_2 , and N_2 , the helium carrier gas flow rate was 3 ml/min, and the temperature program was: initial 30°C hold 6 min, increase to 80°C at 15°C/min, hold 4 min. To determine carbon dioxide concentration (CO_2), the helium carrier gas flow rate was 50 ml/min and the temperature program was: initial 60°C, increase to 250°C at 20°C/min, hold 6 min. To determine the concentration of H_2 and He, the argon carrier gas flow rate was 2 ml/min and temperature program was: initial 10°C hold 10 min, increase to 80°C at 25°C/min, hold 7 min. All analyses were run in triplicate and mean values are reported. Reproducibility for triplicate analyses was $\pm 5\%$.

Stable carbon and hydrogen isotopic analysis for all hydrocarbons was performed at the Stable Isotope Laboratory at the University of

Toronto. Analyses for $\delta^{13}C$ values were performed by gas chromatograph-combustion-isotope ratio mass spectrometry (GC-C-IRMS) with a Finnigan MAT 252 mass spectrometer interfaced with a Varian 3400 capillary GC. Hydrocarbons were separated by a Poraplot QTM column (25 m x 0.32 mm ID) with the following temperature program: initial 40°C hold 1 min, increase to 190°C at 5°C/min, hold 5 min. To separate CO_2 from CH_4 the program was started at 10°C (hold 2 min) increased to 190°C at 5°C/min (hold 5 min). Total error incorporating both accuracy and reproducibility was $\pm 0.5\%$ with respect to V-PDB standard. The detection limit for $\delta^{13}C$ analysis for this analytical setup is a m/z 44 signal size of 0.3 V.

The δ^2H analysis was performed on a continuous flow compound specific hydrogen isotope mass spectrometer that consists of an HP 6890 gas chromatograph (GC) interfaced with a micro-pyrolysis furnace (1465°C) in line with a Finnigan MAT Delta⁺-XL isotope ratio mass spectrometer. The hydrocarbon gases were separated on a Poraplot QTM column (25 m x 0.32 mm ID) with a helium carrier at 2.2 ml/min and the following temperature program: initial 35°C hold 3 min, increase to 180°C at 15°C/min. Total error incorporating both accuracy and reproducibility was $\pm 5\%$ with respect to V-SMOW. The detection limit for δ^2H analysis for this analytical setup is a m/z 2 signal size of 2 V.

The hydrogen and oxygen isotopic analyses of waters were performed at the Environmental Isotope Laboratory, University of Waterloo, Waterloo, Canada. $\delta^2H_{H_2O}$ was determined by manganese reduction at 900°C using a method modified from Coleman et al. (1982). $\delta^{18}O_{H_2O}$ analyses were performed by the CO_2 equilibration method of Epstein and Mayeda (1953) and Fritz et al. (1986). Reproducibility on duplicate analyses are $\pm 0.4\%$ and $\pm 0.1\%$ with respect to V-SMOW/SLAP, for δ^2H and $\delta^{18}O$ respectively.

For the PCR-amplification of archaeal-domain small subunit ribosomal RNA genes (SSU rDNAs), total DNA was extracted from filters collected from packered boreholes as previously described (Moser et al., 2002), using the Ultra-Clean Soil kit (MoBio, CA) according to the manufacturer's protocol at Princeton and Portland State Universities.

Table 1. Gas compositions (in %) from sites in the Witwatersrand basin, and groundwater and gas flow rates (in L/min).

Mine	Fm	Borehole	Ar	H ₂	He	O ₂	N ₂	CH ₄	C ₂ H ₆	C ₃ H ₈	C ₄ H ₁₀	N ₂ /O ₂	CH ₄ / C ₂ +	Water flow	Gas flow	T °C
Beatrix	Wits	BE16 DW hole 1	0.60	<0.01	0.70	5.04	69.7	22.3	<0.01	<0.01	<0.01	13.8	na	10	0.33	34
Beatrix	Wits	BE16 GDW 1	0.84	<0.01	1.06	15.4	67.2	17.8	0.18	<0.01	<0.01	4.4	98.9	1.2	0.06	34
Beatrix	Wits	BE16 GDW hole 2	0.94	<0.01	7.53	6.70	24.1	65.3	0.07	<0.01	<0.01	3.6	933	1.2	0.06	NM
Beatrix	Wits	BE16 EDW 1	0.27	<0.01	1.88	2.54	7.76	89.6	0.17	<0.01	<0.01	3.1	527	NM	30	NM
Beatrix	Wits	BE23 A4rd	0.92	<0.01	0.56	17.5	72.8	10.2	3.01	<0.01	<0.01	4.2	3.39	5	3	34
Beatrix	Wits	BE24 C18W17	0.96	<0.01	0.20	19.7	75.3	7.73	<0.01	<0.01	<0.01	3.8	na	NM	NM	33
Beatrix	Wits	BE339 CTS 1	1.05	<0.01	12.6	0.71	19.5	69.7	0.14	0.02	<0.01	27.4	436	38	40	39
Beatrix	Wits	BE339 CTS 2	1.01	<0.01	13.5	5.10	19.7	65.8	0.13	<0.01	<0.01	3.9	506	NM	0.67	NM
Beatrix	Wits	BE341 CTS 1	0.98	<0.01	12.2	0.57	19.3	69.2	0.06	<0.01	<0.01	34.0	1150	NM	NM	40
Masimong	Wits	MM5 18702SW	NM	<0.01	7.80	8.25	39.1	40.6	0.02	<0.01	<0.01	4.7	2030	NM	NM	42
Merriespruit	Wits	MP1 51XCTEAST	NM	<0.01	4.10	11.7	48.0	26.5	0.02	<0.01	<0.01	4.1	1325	NM	NM	39
Evander	Wits	EV219 ED2 hole 1	0.54	<0.01	7.57	0.99	35.2	62.6	0.28	<0.01	<0.01	35.6	224	0.12	0.15	31
Evander	Wits	EV219 ED1 hole 5	0.93	<0.01	0.07	20.1	84.8	1.47	<0.01	<0.01	<0.01	4.2	na	0.58	0.9	32
Evander	Wits	EV552 HWD 1	0.65	<0.01	5.64	2.40	48.2	49.2	0.72	<0.01	<0.01	20.1	68.3	11	0.25	NM
Evander	Wits	EV522 CTS hole 1	0.57	<0.01	2.77	0.54	20.8	78.8	3.34	0.33	0.04	38.5	21.3	0.7	0.1–0.2	32
Evander	Wits	EV522 CTS hole 2	0.54	<0.01	3.10	0.55	17.6	77.3	3.47	0.34	0.05	31.8	20.0	1.8	0.2–0.5	36
Evander	Wits	EV818 NEPD 1	0.69	0.01	9.96	0.59	20.7	62.0	3.10	0.26	0.03	35.1	18.3	10–15	7.5	45
Evander	Wits	EV818 NEPD	0.72	<0.01	3.42	2.90	25.0	57.3	2.68	0.24	0.03	8.6	19.4	1.2	2.4	45
Kloof	Vent	KL441XC56HWD S hole 2	1.68	<0.01	15.6	6.55	61.9	23.4	0.51	0.05	<0.01	9.4	41.8	18	0.21	56
Kloof	Vent	KL443 HWNW 1	1.39	<0.01	18.2	1.24	13.3	57.3	2.03	0.28	0.05	10.7	24.3	1.5	0.95	NM
Kloof	Vent	KL443 HWDN	1.79	<0.01	20.8	0.97	16.8	53.4	2.04	0.21	0.03	17.3	23.4	22	2.6	59
Middelbult	Karoo	MB Sec49	0.16	<0.01	<0.01	0.69	13.1	93.0	<0.01	<0.01	<0.01	18.9	na	30–40	2.9	27
Middelbult	Karoo	MB R531005	0.14	<0.01	0.04	0.89	9.55	94.5	<0.01	<0.01	<0.01	10.7	na	NM	NM	NM
Middelbult	Karoo	MB W135704-1	0.13	<0.01	<0.01	0.68	8.09	93.7	<0.01	<0.01	<0.01	11.9	na	NM	NM	NM
Middelbult	Karoo	MB W135704-2	0.35	<0.01	<0.01	6.07	27.7	73.1	<0.01	<0.01	<0.01	4.6	na	NM	NM	NM
Middelbult	Karoo	MB W135035	0.09	<0.01	0.10	0.74	4.92	82.9	<0.01	<0.01	<0.01	6.7	na	NM	NM	NM

All CO₂ concentrations were <d.l.

na = CH₄/C₂+ ratios not available due to C₂+ concentrations <d.l.

NM = not measured.

Fifty- μ L reactions were employed to amplify SSU rDNAs using *Ex Taq* polymerase (TaKaRa, Kyoto, Japan), the provided buffers and dNTPs, and oligonucleotide primers at 0.4 μ M and template 50 ng/ μ L. SSU rDNAs were amplified with the primers, Arch21F (archaea-specific, 5'-CTG GTT GAT CCT GCC AG-3', (DeLong, 1992)) and U1492r (universal, 5'-CGG TTA CCT TGT TAC GAC TT-3', (Lane, 1991)). In samples where the first amplification failed to yield a detectable product, nested amplifications were performed using the archaeal-specific primers, S-D-Arch-0344-a-S-20 (5'-ACG GGC CGC AGC AGG CGC GA-3', (Raskin et al., 1994)) and 958r (5'-TCC GGC GTT GAM TCC AAT T-3', (DeLong, 1992)). PCR was performed for 30 cycles: denaturation for 30 s at 94°C, annealing for 45 s at 54°C, and elongation for 120 s at 72°C. After verification by agarose gel electrophoresis, products were purified and cloned into pCR2.1 using the TOPO TA cloning kit (Invitrogen, CA) following the manufacturer's protocol. At least 40 cloned colonies were picked and reamplified with M13R and T7 primers, and PCR products were subsequently digested with HaeIII, HhaI, and RsaI restriction enzymes for restriction fragment length polymorphism (RFLP) analyses. Clones with distinctive RFLP types were chosen for complete sequencing. Three sequencings were applied to the most dominant RFLP type. Clones represented by each unique RFLP type were assumed to contain the same sequence. The sequences were compiled and aligned with sequences obtained from GenBank and the RDP database II using ARB software (Strunk and Ludwig, 1995; Maidak et al., 2001). A distance matrix for Neighbor Joining analysis was calculated according to the algorithm of Jukes and Cantor (1969) using the PHYLIP program (Felsenstein, 1993). The Parsimony and Maximum Likelihood trees were constructed by using the parsimony subprogram within ARB and FASTDNAML (Felsenstein, 1993), respectively. Bootstrap values were calculated on the basis of 1000 iterations. Neighbor Joining, parsimony, Maximum Likelihood, and bootstrap analyses were performed on at least 580 homologous bases. All three produced the same branch pattern.

4. RESULTS AND DISCUSSION

The gases from the Karoo Formation at Middelbult mine are coalbed gases consisting primarily of CH₄ with smaller amounts of N₂ (Table 1). While most samples had O₂ concentrations <1%, one sample (MB W135704–2) had an O₂ concentration of 6.07% (and a N₂/O₂ ratio close to 4), reflecting a higher degree of air in this sample and a concomitant decrease in the CH₄ concentration to 73.1% due to dilution with air. If the assumption is made that all O₂ is due to air contamination, compositional results can be “corrected” by subtracting the amount of O₂ and a corresponding amount of N₂, Ar, CO₂, and renormalizing the corrected volume % values for all the other gases. If the sample MB W135704–2 is corrected in this way, its composition becomes very similar to the 4 others from this site. Such a correction is not routinely applied to samples in this study; however, since all O₂ can not be attributed to contamination during sampling. High O₂ concentrations (i.e., ~20%) were occasionally observed even at boreholes with relatively high gas flow rates (>0.9 L/min) that would make significant air contamination during sampling unlikely. This phenomenon suggests that at some boreholes the groundwater is already saturated with respect to air before sampling. Atmospheric air has a N₂/O₂ ratio of 4, therefore, for samples with N₂/O₂ close to 4, both O₂ and N₂ can be assumed to be primarily atmospheric in origin, either due to air contamination during sampling or due to air entrainment in groundwater (Table 1).

For all the other samples from Middelbult mine, CH₄ con-

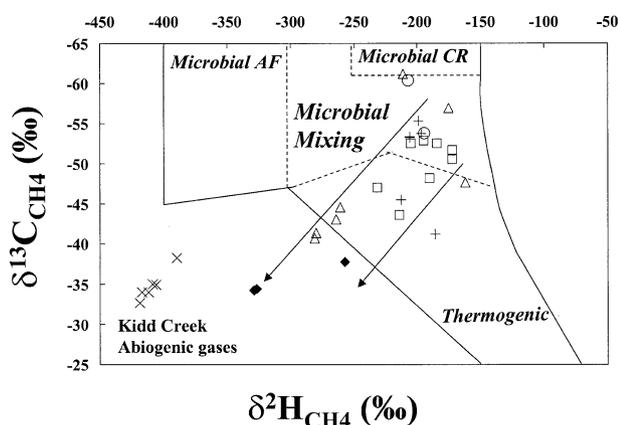


Fig. 3. $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values for all samples compared to empirically determined fields for thermogenic gas and microbial gas produced by CO_2 reduction (CR) and acetate fermentation (AF) after Whiticar (1999). Samples from the Karoo formation (Middelbult mine) are indicated by (+). All samples from the Witwatersrand Supergroup are open symbols, and all samples from the Ventersdorp Supergroup are solid symbols. Symbols from individual mines are as follows: Merriespruit and Masimong (circles); Beatrix (squares); Evander (triangles); Kloof (diamonds). Kidd Creek data (X) are for abiogenic methane from Sherwood Lollar et al. (2002b). Error bars are $\pm 0.5\%$ for $\delta^{13}\text{C}_{\text{CH}_4}$ and $\pm 5\%$ for $\delta^2\text{H}_{\text{CH}_4}$ and are smaller than the plotted symbols. Arrows indicate that samples from Beatrix and Evander fall along apparent mixing trends between a microbial CH_4 component and a more ^{13}C -enriched and ^2H -depleted end member.

concentrations range from 82.9 to 94.5%, with no detectable C_2+ (ethane, propane or butane). Small amounts of ethane, propane, and butane are often formed during gas production from coal,

but they are generally so strongly adsorbed to the coal that their true yields are difficult to quantify (Hunt, 1996). Although a theoretical value for CH_4/C_2+ could be calculated for these gases based on the detection limit ($<0.01\%$) for C_2+ gases, these values might not reflect formation processes but rather postformation loss of C_2+ due to adsorption or other processes, so such theoretical calculations are not standard practice (Hunt, 1996).

For samples from both the Witwatersrand and Ventersdorp Supergroup formations, CH_4 concentrations were typically between 50 to 90%, except in samples for which O_2 concentrations were particularly high due to contamination during sampling or to air entrainment in groundwater (Table 1). In all cases the two other major components in the gases are N_2 and He.

Figure 3 shows typical ranges of stable isotope values for methane produced by microbial and by thermogenic processes based on empirical data (Whiticar, 1999). Carbon and hydrogen isotope values for CH_4 are a well-established means of distinguishing between methane produced by thermogenic versus microbial processes, providing the isotopic values have not suffered secondary isotopic exchange or fractionation (Schoell, 1980; Schoell, 1988). They can also be used to distinguish between microbial CH_4 production by acetate fermentation (AF) versus CO_2 reduction (CR) (Whiticar et al., 1986). The majority of the samples from Merriespruit, Masimong, Beatrix, and Evander mines (Witwatersrand Supergroup) fall within the range of microbially produced CH_4 or microbial mixing with a possible thermogenic component (Table 2 and Fig. 3). For Beatrix and Evander, in particular, samples fall along trends that suggest mixing between a ^{13}C -depleted microbial CH_4 component and a more ^{13}C -enriched and ^2H -depleted end member. In contrast, samples from the Ventersdorp Supergroup

Table 2. Carbon and hydrogen isotopic values (in ‰).

Mine	Formation	Borehole	$\delta^{13}\text{C}_{\text{CH}_4}$	$\delta^2\text{H}_{\text{CH}_4}$	$\delta^{13}\text{C}_{\text{C}_2\text{H}_6}$	$\delta^2\text{H}_{\text{C}_2\text{H}_6}$	$\delta^{13}\text{C}_{\text{C}_3\text{H}_8}$	$\delta^2\text{H}_{\text{C}_3\text{H}_8}$	$\delta^{13}\text{C}_{\text{C}_4\text{H}_{10}}$	$\delta^2\text{H}_{\text{C}_4\text{H}_{10}}$
Beatrix	Wits	BE16 DW hole 1	-48.1	-190	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE16 GDW 1	-51.6	-172	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE16 GDW hole 2	-52.5	-205	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE16 EDW 1	-43.5	-214	-27.1	-221	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE23 A4rd	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE24 C18W17	-47.0	-231	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE339 CTS 1	-52.8	-195	-38.2	-183	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE322 CTS 2	-52.5	-184	-36.3	-232	<d.l.	<d.l.	<d.l.	<d.l.
Beatrix	Wits	BE341 CTS 1	-50.5	-172	-38.2	-212	<d.l.	<d.l.	<d.l.	<d.l.
Masimong	Wits	MM5 18702SW	-60.7	-207	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Merriespruit	Wits	MP1 51XCTEAST	-53.7	-194	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Evander	Wits	EV219 ED 2 hole 1	-56.9	-175	-34.8	-140	<d.l.	<d.l.	<d.l.	<d.l.
Evander	Wits	EV219 ED1 hole 5	-61.2	-211	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Evander	Wits	EV552 HWD 1	-47.6	-162	-24.6	-138	<d.l.	<d.l.	<d.l.	<d.l.
Evander	Wits	EV522 CTS hole 1	-43.0	-264	-35.0	-215	-33.7	-183	-33.6	-161
Evander	Wits	EV522 CTS hole 2	-44.5	-261	-39.5	-238	-33.3	-176	-33.8	-157
Evander	Wits	EV818 NEPD 1	-40.7	-281	-31.7	-257	-34.0	-180	<d.l.	<d.l.
Evander	Wits	EV818 NEPD	-41.4	-280	-31.7	-233	-34.6	-175	-33.4	-158
Kloof	Vent	KL441XC56HWDS hole 2	-37.8	-257	-32.9	-198	-34.0	-105	<d.l.	<d.l.
Kloof	Vent	KL443 HWND 1	-34.4	-327	-35.6	-251	-33.7	-208	<d.l.	<d.l.
Kloof	Vent	KL443 HWND	-34.3	-329	-34.3	-269	-33.5	-206	-33.9	-200
Middelbult	Karoo	MB Sec49	-45.5	-213	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Middelbult	Karoo	MB R531005	-53.2	-206	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Middelbult	Karoo	MB W135704-1	-53.7	-197	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Middelbult	Karoo	MB W135704-2	-53.3	-206	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Middelbult	Karoo	MB W135035	-55.3	-199	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.

<d.l. = below detection limit for isotope analysis.

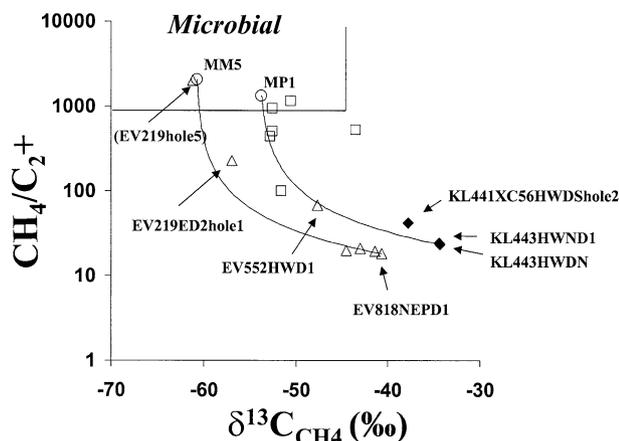


Fig. 4. CH_4/C_2^+ versus $\delta^{13}\text{C}_{\text{CH}_4}$ values for mixing with microbial hydrocarbon gas after Hunt (1996). Mixing lines are calculated as in the text and provide a conservative estimate of microbial CH_4 contributing to the various samples (see text). Symbols are as in Figure 3. Errors on $\delta^{13}\text{C}_{\text{CH}_4}$ values are $\pm 0.5\text{‰}$, and errors on CH_4/C_2^+ are $\pm 7\%$. All error bars are smaller than the plotted symbols. The Karoo coalbed gases from Middelbult mine do not appear since there is no detectable C_2^+ in these samples (see text).

(Kloof mine—solid diamonds) are significantly more ^{13}C -enriched and ^2H -depleted than the Witwatersrand Supergroup and Karoo formation gases (Fig. 3).

4.1. Microbial Origin of ^{13}C -Depleted End Member

Figure 4 provides further support for mixing between microbial CH_4 and a more ^{13}C -enriched CH_4 end member. Typical

microbial hydrocarbon gas has a $\delta^{13}\text{C}_{\text{CH}_4}$ value more depleted than -45‰ , with CH_4/C_2^+ ratios $\geq 10^3$ (Hunt, 1996). As in Figure 3, the samples from Merriespruit and Masimong (MM5 and MP1) are most consistent with a microbial gas end member, with $\delta^{13}\text{C}$ values of -60.7 and -53.7‰ , respectively, and CH_4/C_2^+ ratios >1000 . Due to the fact that C_2^+ concentrations for EV219hole5 are $<$ detection limit, no CH_4/C_2^+ ratio can be calculated for this sample. The low C_2^+ concentrations are likely due to air contamination in this sample (N_2/O_2 ratio = 4.2) causing a decrease in the observed concentration of CH_4 and dilution of C_2^+ peaks to $<$ detection limit. This dilution does not affect $\delta^{13}\text{C}$ values however, so the $\delta^{13}\text{C}_{\text{CH}_4}$ value for EV219hole5 (-61.2‰) is shown in Figure 4 in brackets. The similarity in $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values between MM5 and EV219hole5 supports a predominantly microbial origin via CO_2 reduction for both these samples (Figs. 3 and 4). In support of this conclusion, EV219hole5 produced SSU rDNA clones related to the autotrophic *Methanosarcina* spp. (*M. mazei*, *M. barkeri*; (Rouviere et al., 1992; Joulain et al., 1998)), as well as *Methanobacterium* spp. (autotrophic; (Whittman et al., 1999)) (Fig. 5). *Methanobacterium* (*M. subterraneum*; (Kotelnikova et al., 1998)) has been previously cultivated from the deep subsurface. rDNA clones related to *Methanosaeta thermoacetophila* and *M. concilii* (exclusively acetotrophic; (Kamagata et al., 1992)), were also obtained from E219 (Fig. 5). No sample was available from MM5 or MP1, but a sample from MP149 from the same fracture zone as MP1 also produced SSU rDNA clones related to methanogens (Fig. 5).

The majority of the data, then, from Beatrix and Evander are consistent with mixing of a microbial end member such as those represented by MM5, MP1, and EV219hole5, with more

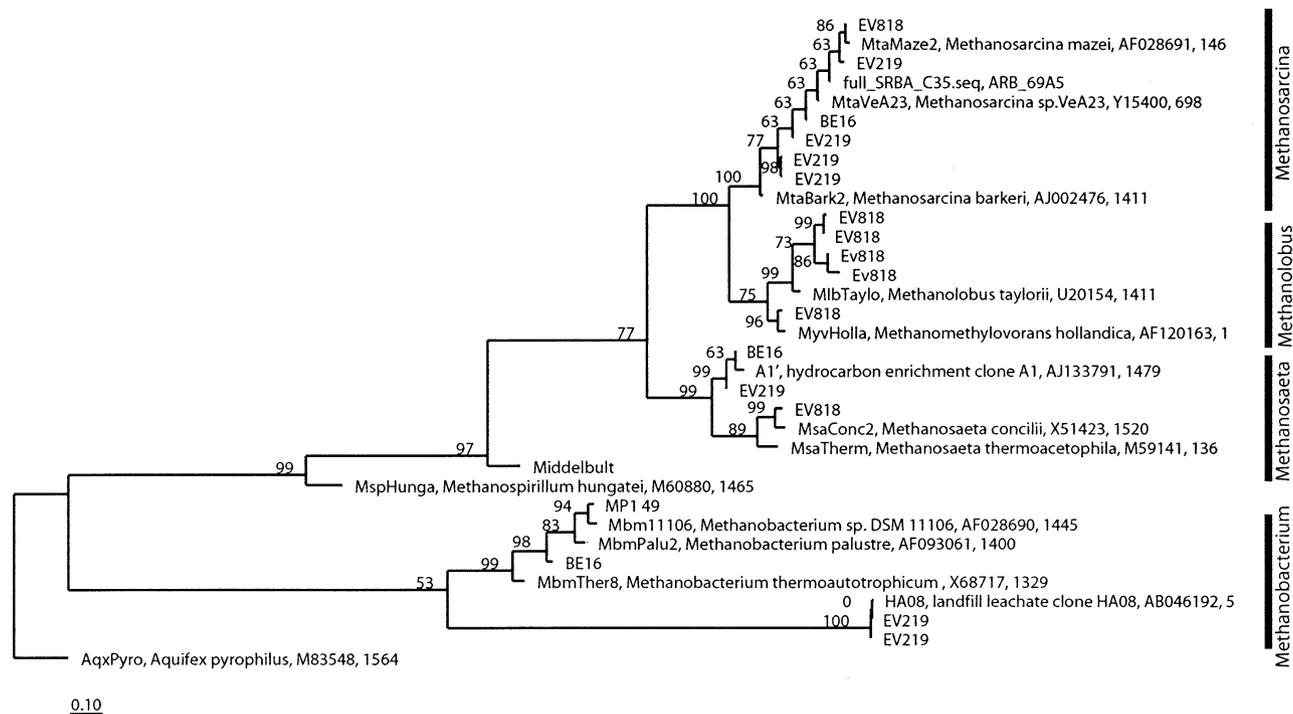


Fig. 5. Phylogenetic dendrogram based on a maximum likelihood analysis (Phylip FastDNAM1) of unambiguously aligned positions of 16S rRNA sequences. The scale bar represents 0.1 changes per nucleotide.

^{13}C -enriched gases with higher C_2+ concentrations (lower CH_4/C_2+ ratios). Although a variety of possible mixing lines can be drawn on Figure 4, the mixing lines depicted bracket most of the samples and can provide an estimate of the amount of microbial CH_4 in each sample. Based on a microbial end member similar to MP1, and assuming mixing with a ^{13}C -enriched end member similar to KL443HWDN, the Beatrix samples are all >90% microbial in origin, and EV552HWD1 is approximately 68% microbial. The most ^{13}C -depleted Kloof sample (KL441XC56HWDShole2) is 18% microbial, based on this calculation. Based on mixing between a microbial end member similar to MM5 and the most ^{13}C -enriched sample at Evander (EV818NEPD1), the Evander samples range from 5% microbial CH_4 (EV818NEPD) to 80% microbial CH_4 for EV219ED2hole1. These estimates are insensitive to whether the CH_4/C_2+ ratios for the microbial end members are close to 10^3 , or even higher. They are, however, dependent on the selected $\delta^{13}\text{C}$ values for the two end members, and hence provide only a conservative estimate of the relative importance of the microbial component in these samples. In particular, the two mixing lines depicted in Figure 4 assume that the most ^{13}C -enriched end member observed at Evander (EV818NEPD1) and at Kloof (KL443HWDN) are entirely free of a microbial contribution. If these samples are themselves the product of mixing with microbial CH_4 with an even more ^{13}C -enriched end member, then estimates of the % microbial contribution would be even larger than those provided above.

Several lines of evidence in fact suggest that the most ^{13}C -enriched samples are not pure end members but do already contain some contribution from microbial CH_4 . The molecular and microbiological data indicate the presence of methanogenic archaea in at least some of the boreholes from Merriespruit, Beatrix, and Evander Mines (Fig. 5). In addition, enrichment cultures have identified active methanogenic populations at Merriespruit (Bonin and Boone, 2004). Further, rDNAs related to those of methanogenic archaea (*Methanosarcina*, *Methanobolus*, *Methanosaeta*, and *Methanobacterium*) indicate the presence of methanogens in several Evander samples, including the most ^{13}C -enriched sample EV818NEPD1 (Fig. 5). Hence, the microbiological evidence supports the idea that this sample is not a pure end member but already is the product of some degree of mixing with microbial hydrocarbons. In contrast, rDNA amplifications for the Kloof samples did not yield any evidence of archaea, much less methanogens. This may, however, be due to the very low biomass in these samples, so a small microbial contribution to the Kloof samples cannot be entirely ruled out. The same fissure sampled by KL441XC56HWDShole2 was previously sampled in 1998 from an adjacent borehole (Takai et al., 2001). In the earlier sampling, archaea were detectable using the same DNA extraction and amplification techniques; however, only two lineages, most closely related to *Pyrococcus abyssi* (non-methanogenic), appeared in the clone library (Takai et al., 2001). Thus, the Ventersdorp Supergroup horizons from this mine, while apparently not sterile, support exceedingly weak methanogen populations. The Kloof samples are the deepest (3.3–3.4 kmbls) and hottest (ca. 56–59°C) of this sample set. They are also extremely low in biomass (below detection by flow cytometry, data not shown). Witwatersrand Supergroup rocks are present beneath Kloof Mine 4 Shaft, but very deep in unmined horizons

below the Ventersdorp Supergroup. If methanogenic populations were to inhabit these rocks, they would have to be hyperthermophilic.

4.2. Origin of the Non-microbial ^{13}C -Enriched End Member

Earlier theories regarding the origin of hydrocarbon gases in the Witwatersrand basin suggested production of thermogenic gas by thermal decomposition of high molecular weight precursors in the shale layers and carbon seams of the Witwatersrand Supergroup (Cook, 1998), migration of thermogenic hydrocarbons into the Witwatersrand basin from outside (Spangenberg and Frimmel, 2001), or production of hydrocarbons from oil in fluid inclusions (Dutkiewicz et al., 1998). Such a thermogenic origin is plausible for the ^{13}C -enriched non-microbial end member in this study, given that the isotopic composition of thermogenic CH_4 produced from Archean organic matter is likely to be relatively depleted in ^{13}C compared to Paleozoic and Mesozoic thermogenic gas (Boreham et al., 2001) due to the relative depletion in ^{13}C of Precambrian organic matter (Schoell and Fellmer, 1981). For the samples from EV522 and EV818 in particular, both $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values (Fig. 3) and CH_4/C_2+ ratios (Table 1) are consistent with a thermogenic origin. Hence, the trends from more ^{13}C -depleted and ^2H -enriched microbial end members to more ^{13}C -enriched and ^2H -depleted samples at Beatrix and Evander (Fig. 3) could be attributed to mixing between microbial CH_4 and thermogenic hydrocarbons. Certainly the trends are not consistent with maturity trends which would produce a progressive isotopic enrichment in both ^{13}C and ^2H with increasing maturity. In contrast, the $\delta^2\text{H}$ values for the most ^{13}C -enriched samples from Kloof mine (KL443HWDN1 and KL443HWDN) are considerably more depleted in ^2H than typical thermogenic gases (Fig. 3). A significant depletion in $\delta^2\text{H}$ values compared to typical thermogenic gases has long been noted for CH_4 found in mines at Precambrian Shield sites worldwide and has been suggested to be a characteristic feature of abiogenic CH_4 produced by water-rock alteration reactions in this geologic environment (Sherwood Lollar et al., 1993b; Sherwood Lollar et al., 2002b).

4.3. Possible Abiogenic Origin for ^{13}C -Enriched End Member at Kloof

Analysis of hydrocarbon gases dissolved in saline groundwaters from Kidd Creek mine, Canada, suggested that gases in these Precambrian (2700 Ma) rocks were abiogenic in origin based on a significant depletion in $\delta^2\text{H}$ values compared to thermogenic gases and a characteristic ^{13}C -depletion and ^2H -enrichment between CH_4 and ethane (Fig. 6), consistent with production of higher molecular weight hydrocarbons by polymerization of CH_4 (Sherwood Lollar et al., 2002b). This pattern results from kinetically controlled synthesis of higher molecular weight hydrocarbons from lower ones, owing to the fact that $^{12}\text{CH}_4$ reacts faster than $^{13}\text{CH}_4$ to form chains, so that ^{12}C is more likely to be incorporated into larger hydrocarbon chains; whereas the light (^1H) isotope will be preferentially eliminated in polymerization reactions owing to preferential cleavage of the lighter $^{12}\text{C}-^1\text{H}$ bond versus the $^{12}\text{C}-^2\text{H}$ bond (Sherwood

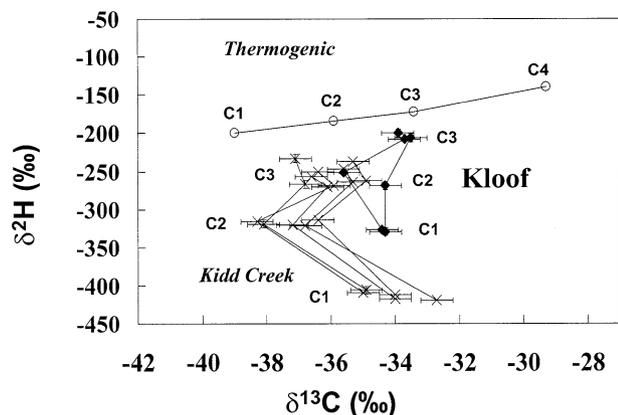


Fig. 6. $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values for CH_4 , ethane, and propane (C1-C3) for the most ^{13}C -enriched samples from Kloof (diamonds). These samples show a pattern of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values between CH_4 and the higher hydrocarbons similar to that described for abiogenic hydrocarbons discovered at Kidd Creek Mine, Timmins, Ontario, Canada (X) (Sherwood Lollar et al., 2002b), and very different from typical thermogenic hydrocarbons (open circles—data from Sherwood Lollar et al., 1994). Error bars are $\pm 0.5\text{‰}$ for $\delta^{13}\text{C}_{\text{CH}_4}$. Error bars are $\pm 5\text{‰}$ for $\delta^2\text{H}_{\text{CH}_4}$ and are smaller than the plotted symbols.

Lollar et al., 2002b). In marked contrast, the typical pattern for thermogenic gas consists of a positive correlation of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values between CH_4 , ethane, and the higher hydrocarbon gases (propane and butane) (Fig. 6). This pattern of increasing isotopic enrichment in both ^{13}C and ^2H with increasing molecular weight for the C_1 - C_4 homologues for thermogenic gases results from production of hydrocarbons by thermal cracking of a high molecular weight organic precursor (Des Marais et al., 1981; Schoell, 1988).

While the samples from EV522 and EV818 show an isotopic enrichment in ^{13}C and ^2H between CH_4 and ethane consistent with a thermogenic origin (Table 2), the most ^{13}C -enriched Kloof samples (KL443HWND1 and KL443HWDN) show a pattern in Figure 6 more consistent with the proposed abiogenic pattern first described at Kidd Creek (Sherwood Lollar et al., 2002b). While an abiogenic origin has been suggested for a number of hydrocarbon occurrences worldwide (Gold, 1979; Abrajano et al., 1990; Charlou and Donval, 1993; Jenden et al., 1993; Kelley et al., 2001), Sherwood Lollar et al. (2002b) was the first to propose that the pattern of ^{13}C depletion and ^2H enrichment between CH_4 and ethane could be used to identify abiogenic synthesis of higher hydrocarbons via polymerization from a CH_4 precursor. Subsequently abiogenic hydrocarbon gases similar to those first identified at Kidd Creek have been described at four other Precambrian Shield sites in Canada and South Africa (Sherwood Lollar et al., 2002a). Potential mechanisms for abiogenic gas synthesis include: surface-catalyzed polymerization from reduction of CO in the Fischer-Tropsch synthesis (Anderson, 1984); heating or metamorphism of graphite-carbonate-bearing rocks (Giardini and Salotti, 1968; Holloway, 1984); or other vapor-water-rock alteration reactions in the presence of catalytically active metals (McCullom and Seewald, 2001). Confirmation of the exact mechanism responsible for the gases in the Ventersdorp lavas is not possible at this point, but with the exception of serpentinization any of the above processes are feasible in this geologic environment.

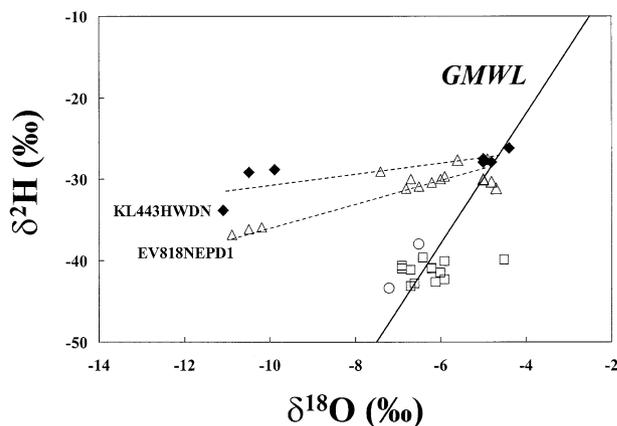


Fig. 7. $\delta^2\text{H}_{\text{H}_2\text{O}}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ for fissure water samples for all sites. The global meteoric waterline (GMWL) is from Craig (1961); I. A. E. A (1981). Possible mixing lines emphasize the trends between samples that lie on the meteoric waterline, and samples that fall above the meteoric waterline. Error bars are based on reproducibility of duplicate analyses and are $\pm 0.4\text{‰}$ and $\pm 0.1\text{‰}$ for $\delta^2\text{H}_{\text{H}_2\text{O}}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, respectively. They are smaller than the plotted symbols. Symbols are as in Figure 3.

4.4. Mixing Constraints Based on $\delta^{18}\text{O}$ and $\delta^2\text{H}$ Values

Whether the Witwatersrand and Ventersdorp formations are host rocks (gases formed *in situ*) or reservoir rocks (where gases are trapped and stored) is an open question. Certainly the abundance of semivertical cross-cutting dykes and fractures throughout the Witwatersrand basin suggest the potential for gas migration and transport along these structures (Fig. 2). $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values measured for borehole waters from the sites in this study (Fig. 7) also support mixing. In an earlier study of fissure waters from the mines of the Witwatersrand basin, Lippmann et al. (2003) measured $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values that largely fell along the meteoric waterline. Both ^{36}Cl ages and residence times based on nucleogenic noble gases indicated that these waters were geologically old (1.5–129 Ma) rather than recent meteoric waters, and supported long distance vertical migration along water-bearing fractures, faults, and dykes (Lippmann et al., 2003). While Lippmann et al. (2003) also identified two samples of more highly saline groundwater with $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values that fell above the meteoric waterline (21–168 Ma in age based on nucleogenic noble gas residence times), the present paper is the first to demonstrate that fissure waters in the Witwatersrand basin are, in fact, controlled by mixing between two distinct end members (Fig. 7). Fissure waters from the Witwatersrand formation at Beatrix, Merriespruit, and Masimong largely fall on the global meteoric waterline (GMWL). Clearly, the Witwatersrand Supergroup hydrocarbon gas samples with the largest microbial component are associated with groundwaters of paleo-meteoric origin. In contrast, samples from Kloof and Evander range from the GMWL to samples falling significantly above the meteoric waterline, consistent with the effects of water-rock interaction such as alteration of feldspar to clay under low water to rock ratios (Onstott et al., 1997), and consistent with $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values found for saline groundwaters from other Precambrian Shield sites worldwide (Frape and Fritz, 1987). As was seen for

the hydrocarbon gas isotopic signatures, the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values for the Kloof and Evander samples are consistent with mixing, in this case between the paleo-meteoric, moderately saline waters with temperatures of 30 to 40°C, and isotopically enriched end members that fall above the meteoric waterline associated with hotter (45–59°C), more saline groundwaters. Significantly, the most saline and isotopically enriched Evander fissure water is also the sample that contains the most ^{13}C -enriched CH_4 (EV818NEPD1; Figs. 4 and 7). Likewise, the samples with the largest microbial CH_4 component (EV219hole5, EV219ED2hole1, and EV552HWD1) lie closest to the meteoric waterline (Fig. 7), while samples EV522CTshole1 and EV522CTshole2 lie in-between these two extremes, just as they do on the mixing lines in Figure 4. Kloof mine samples show a similar distribution with the most ^{18}O - and ^2H -enriched isotopic groundwaters corresponding to the most ^{13}C -enriched CH_4 sample—KL443HWDN. In contrast, the sample with the most significant microbial component (KL441 HWDShole2) has $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values falling on the meteoric waterline.

5. SUMMARY AND IMPLICATIONS FOR THE DEEP BIOSPHERE

Compositions and $\delta^{13}\text{C}$ and $\delta^2\text{H}$ isotopic values for hydrocarbon gases from mines in the Witwatersrand basin, South Africa, support the widespread occurrence of microbially produced hydrocarbon gas in millions of years-old fissure waters. The presence of microbial hydrocarbons is, to a large extent, controlled by the geologic formations in which the gases are found. Samples from the Witwatersrand Supergroup have the largest component of microbial gas, while samples from the Ventersdorp Supergroup support only a smaller component of microbial CH_4 (KL441XC56HWDShole2). At all sites, the distribution of $\delta^{13}\text{C}$ values and CH_4/C_2+ ratios are consistent with mixing between microbial CH_4 , and a more ^{13}C -enriched and C_2+ -rich end member. While samples from Evander mine are not inconsistent with a thermogenic origin for the ^{13}C -enriched end member, samples from Kloof mine require an additional hydrocarbon source similar in ^{13}C and ^2H isotopic patterns to gases found at other Precambrian Shield sites (Sherwood Lollar et al., 2002a; Sherwood Lollar et al., 2002b). Although a microbial component cannot be ruled out for the Karoo coalbed gases from Middelbult mine, the $\delta^{13}\text{C}$ values and high CH_4 content with relatively small amounts of N_2 are consistent with production of gas from relatively immature organic source matter (Hunt, 1996) and do not require invoking a microbial contribution.

Mixing trends identified based on hydrocarbon gas compositions and isotopic values are confirmed by $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values measured for fissure waters from the same boreholes. Paleowaters of meteoric origin with temperatures of 30 to 40°C are associated with samples with the largest component of microbial hydrocarbon gas. Samples from the Witwatersrand Supergroup from Beatrix, Merriespruit, and Masimong mines contain the largest component of this paleo-meteoric, microbial end member. In contrast, samples from Evander and Kloof mines reflect mixing between samples with microbial hydrocarbon gases and meteoric $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values, and a component of more ^{13}C -enriched hydrocarbon gases of non-micro-

bial origin dissolved in the more saline, ^{18}O - and ^2H -enriched, hotter (45–59°C) fissure waters.

While some mixing of the fissure waters with a small component of modern meteoric water introduced due to mining operations cannot be excluded, the 100 Ma ages established by Lippmann et al. (2003) for these fissure waters suggests that both the Witwatersrand and Ventersdorp end members are geologically old groundwaters stored over long periods in hydrogeologically isolated fracture-controlled flow systems. The case for the existence of indigenous microbial communities in both the Witwatersrand (Takai et al., 2001) and Ventersdorp Supergroup (Takai et al., 2001; Baker et al., 2003; Moser et al., 2003b) is growing. Microbial communities in flowing boreholes, in several cases, have been shown to contain no common SSU rDNA clones in comparisons of libraries from both fissure waters and known sources of contamination (mine service water and air (Baker et al., 2003; Moser et al., 2003b; Onstott et al., 2004). The apparently great age and hydrologic isolation (Takai et al., 2001; Lippmann et al., 2003; Moser et al., 2003b) of the Ventersdorp Supergroup rocks may be consistent with the report of molecular evidence for possible hyperthermophilic organisms related to *Pyrococcus Abyssii* (Takai et al., 2001) in hot waters (50–60°C) from the Ventersdorp Supergroup. The substantial component of microbial hydrocarbons identified in samples from the Witwatersrand Supergroup in this study, and the determination of SSU rDNA sequences related to methanogens from some of these same boreholes, support the widespread occurrence of methanogenic microbial communities in the deep subsurface of the Witwatersrand basin. Stable isotope and gas geochemistry can provide a powerful approach for documenting the presence of such communities in the subsurface.

Acknowledgments—This study was supported in part by the Natural Sciences and Engineering Research Council of Canada and by the National Science Foundation Life in Extreme Environments Program (EAR-9714214). We thank H. Li for compositional and isotopic analyses. Special thanks are due to the geologists and staff of the following mines for providing geological information and invaluable assistance with underground field work: Evander (C. Ralston, W. Seymoor, Harmony Mines), Merriespruit (J. Larkan and V. Sewpersad, Harmony Mines), Masimong (J. Bashoff, Harmony Mines), Mponeng (D. Kershaw and G. Gilchrest), Kloof (A. van Heerden, D. Steinkamp, T. Hewitt, G. Buxton). Special thanks to Rob Wilson of SRK-Turgis for logistical support.

Associate editor: R. Summons

REFERENCES

- Abrajano T. A., Sturchio N. C., Bohlke J. K., Lyon G. L., Poreda R. J., and Stevens C. M. (1988) Methane-hydrogen gas seeps, Zambales Ophiolite, Philippines: deep or shallow origin? *Chem. Geol.* **71**, 211–222.
- Abrajano T. A., Sturchio N., Kennedy B. M., Lyon G. L., Muehlenbachs K., and Bohlke J. K. (1990) Geochemistry of reduced gas related to serpentinization of the Zambales ophiolite, Philippines. *Applied Geochemistry* **5**, 625–630.
- Anderson R. B. (1984) *The Fischer-Tropsch Synthesis*. Academic Press.
- Baker B. J., Moser D. P., MacGregor B. J., Fishbain S., Wagner M., Fry N. K., Jackson B., Speolstra N., Loos S., Takai K., Sherwood Lollar B., Fredrickson J. K., Balkwill D., Onstott T. C., Wimpee C. F., and Stahl D. A. (2003) Related assemblages of sulphate-reducing bacteria associated with ultradeep gold mines of South Africa and deep

- basalt aquifers of Washington State. *Environmental Microbiology* **5**, 267–277.
- Berndt M. E., Allen D. E., and Seyfried W. E. J. (1996) Reduction of CO₂ during serpentinization of olivine at 300C and 500 bar. *Geol.* **24**, 351–354.
- Bonin A. S. and Boone D. R. (2004) Microbial isolations and characterizations from the deep terrestrial subsurface of the South African gold mines. Proceedings of the 104th General Meeting of the American Society for Microbiology. New Orleans, LA. May 2004.
- Boreham C. J., Hope J. M., and Hartung-Kagi B. (2001) Understanding source, distribution and preservation of Australian natural gas: A geochemical perspective. *Australian Production and Petroleum Exploration Association Journal* **43**, 523–547.
- Charlou J. L. and Donval J. P. (1993) Hydrothermal methane venting between 12N and 26N along the Mid-Atlantic ridge. *J. of Geophysical Research* **98**, 9625–9642.
- Coleman M. L., Shepherd T. J., Durham J. J., Rouse J. E., and Moore G. R. (1982) Reduction of water with zinc for hydrogen isotope analysis. *Analytical Chemistry* **54**, 993–995.
- Cook A. P. (1998) The occurrence, emission and ignition of combustible strata gases in Witwatersrand gold mines and Bushveld platinum mines and means of ameliorating related ignition and explosion hazards. In *Safety in Mines Research Advisory Committee, Interim Project Report*, GAP. Vol. **504**, Itasca Africa (Pty) Ltd. 90 pages.
- Coward M. P., Spencer R. M., and Spencer C. E. (1995) Development of the Witwatersrand Basin, South Africa. *Early Precambrian Processes* **95**, 243–269.
- Craig H. (1961) Isotopic variations in meteoric waters. *Science* **133**, 1702–1703.
- DeLong E. F. (1992) Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences* **89**, 5685–5689.
- Des Marais D. J., Donchin J. H., Nehring N. L., and Truesdell A. H. (1981) Molecular carbon isotopic evidence for the origin of geothermal hydrocarbons. *Nature* **292**, 826–828.
- Dutkiewicz A., Rasmussen B., and Buick R. (1998) Oil preserved in fluid inclusions in Archaean sandstones. *Nature* **395**, 885–888.
- Epstein S. and Mayeda T. K. (1953) Variations of the ¹⁸O/¹⁶O ratio in natural waters. *Geochim. Cosmochim. Acta* **4**, 213.
- Eschenburg H. M. W. (1980) Sources and control of methane in gold mines. *J. of the Mine Ventilation Society of South Africa* **33**, 125–135.
- Felsenstein J. (1993) *PHYLIP (Phylogeny inference package)*. University of Washington.
- Frape S. K. and Fritz P. (1987) Geochemical trends from groundwaters from the Canadian Shield. In *Saline Waters and Gases in Crystalline Rocks*, Vol. Special Paper 33 (ed. P. Fritz and S. K. Frape), pp. 19–38, Geological Association of Canada.
- Fritz P., Frape S. K., Drimmie R. J., and Heemskerck A. R. (1986) Reply to comments by Grabczak et al. on Water-rock interaction and chemistry of groundwaters from the Canadian Shield. *Geochim. Cosmochim. Acta* **50**, 1561–1563.
- Fritz P., Frape S. K. and Miles M. (1987) Methane in the crystalline rocks of the Canadian Shield. In *Saline Water and Gases in Crystalline Rocks*, Vol. Special Paper 33 (ed. P. Fritz and S. K. Frape), pp. 211–224, Geological Association of Canada.
- Giardini A. A. and Salotti C. A. (1968) Synthesis of graphite and hydrocarbons by reaction between calcite and hydrogen. *Science* **159**, 317–319.
- Giardini A. A. and Salotti C. A. (1969) Kinetics and relations in the calcite-hydrogen reaction and relations in the dolomite-hydrogen and siderite-hydrogen systems. *American Mineralogist* **54**, 1151–1172.
- Gold T. (1979) Terrestrial sources of carbon and earthquake outgassing. *J. Petrol. Geol.* **1**, 1–19.
- Holloway J. R. (1984) Graphite-CH₄-H₂O-CO₂ equilibria at low grade metamorphic conditions. *Geol.* **12**, 455–458.
- Horita J. and Berndt M. E. (1999) Abiogenic methane formation and isotopic fractionation under hydrothermal conditions. *Science* **285**, 1055–1057.
- Hu G., Ouyang Z., Wang X., and Wen Q. (1998) Carbon isotopic fractionation in the process of Fischer-Tropsch reaction in primitive solar nebula. *Science in China* **41**, 202–207.
- Hunt J. M. (1996) *Petroleum Geochemistry and Geology*. W.H. Freeman and Company.
- I. A. E. A. (1981) *Statistical treatment of environmental isotope data in precipitation*. International Atomic Energy Agency Technical Report Series #206, 255 pages. Vienna, Austria.
- Jenden P. D., Hilton D. R., Kaplan I. R. and Craig H. (1993) Abiogenic hydrocarbons and mantle helium in oil and gas fields. In *The Future of Energy Gases - USGS Professional Paper 1570* (ed. D. G. Howell), pp. 57–82, United States Geological Survey.
- Johnson M. R., van Vuuren C. J., Hegeberger W. F., Key R., and Shoko U. (1993) Stratigraphy of the Karoo Supergroup in southern Africa: an overview. *Journal of African Earth Sciences* **23**, 3–15.
- Joulian C., Ollivier B., Patel B. K. C., and Roger P. A. (1998) Phenotypic and phylogenetic characterization of dominant culturable methanogens isolated from ricefield soils. *FEMS Microbiol. Ecol.* **25**, 135–145.
- Jukes, T. H. and Cantor, C. R. (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. H.N. Munro), pp. 21–132, Academic Press.
- Kamagata Y., Kawasaki H., Oyaizu H., Nakamura K., Mikami E., Endo G., Koga Y., and Yamasato K. (1992) Characterization of three thermophilic strains of *Methanothrix* (“*Methanoseta*”) *thermophila* sp. nov. and rejection of *Methanothrix* (“*Methanoseta*”) *thermoacetophila*. *Int. J. Syst. Bacteriol.* **42**, 463–468.
- Kelley D. S., Karson J. A., Blackman D. K., Fruh-Green G. L., Butterfield D. A., Lilley M. D., Olson E. J., Schrenk M. O., Roe K. K., Lebon G. T., and Rivizzigno P. (2001) An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. *Nature* **412**, 145–149.
- Kenney J. F., Kutcherov V. A., Bendeliani N. A., and Alekseev V. A. (2002) The evolution of multicomponent systems at high pressures: The thermodynamic stability of the hydrogen-carbon system: The genesis of hydrocarbons and the origina of petroleum. *Proceedings of the National Academy of Sciences* **99**, 10976–10981.
- Kieft T. L., Fredrickson J. K., Onstott T. C., Gorby Y. A., Kostandarithes H. M., Bailey T. J., Kennedy D. W., Li S. W., Plymale A. E., Spadoni C. M., and Gray M. S. (1999) Dissimilatory reduction of Fe(III) and other electron acceptors by a *Thermus* isolate. *Applied and Environmental Microbiology* **65**, 1214–1221.
- Kotelnikova S., Macario A. J., and Pedersen K. (1998) *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater. *Int. J. Syst. Bacteriol.* **48**, 357–367.
- Lane, D. J. (1991) 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics* (ed. E. Stackebrandt and M. Goodfellow), pp. 115–175, Wiley.
- Lippmann J., Stute M., Torgersen T., Moser D. P., Hall J., Lin L., Borcsik M., Bellamy R. E. S., and Onstott T. C. (2003) Dating ultra-deep mine waters with noble gases and ³⁶Cl, Witwatersrand Basin, South Africa. *Geochim. Cosmochim. Acta* **67**, 4597–4619.
- Maidak B. L., Cole J. R., Lilburn T. G., Parker C. T., Jr, Saxman P. R., Farris R. J., Garrity G. M., Olsen G. J., Schmidt T. M., and Tiedje J. M. (2001) The RDP-II (Ribosomal Database Project). *Nucleic Acid Res* **29**, 173–174.
- McCollom T. M. and Seewald J. S. (2001) A reassessment of the potential for reduction of dissolved CO₂ to hydrocarbons during serpentinization of olivine. *Geochim. Cosmochim. Acta* **65**, 3769–3778.
- Moser D. P., Martin H. W. and Boston P. J. (2002) Microbiological sampling in caves and mines. In *Encyclopedia of Environmental Microbiology* (ed. G. Bitton), pp. 821–835, John Wiley.
- Moser D. P., Onstott T. C., Fredrickson J. K., Brockman F., Balkwill D., Pffiffer S., White D. C., Takai K., Pratt L. M., Fong J., Sherwood Lollar B., Slater G. F., Phelps T. J., Spoelstra N., DeFlaun M., Southam G., Welty A. T., Baker B. and Hoek J. (2003b) Temporal shifts in microbial community structure and geochemistry of an ultradeep South African gold mine borehole. *Geomicrobiology J.* **20**, 1–32.
- Nivin V. A., Devirts A. L., and Lagutina Y. P. (1995) The origin of the gas phase in the Lovozero Massif based on hydrogen-isotope data. *Geochemistry International* **32**, 65–71.
- Nurmi P. A., Kukkonen I. T., and Lahermo P. W. (1988) Geochemistry and origin of saline groundwaters in the Fennoscandian Shield. *Applied Geochemistry* **3**, 185–203.

- Onstott T. C., Tobin K., Dong H., DeFlaun M. F., Fredrickson J. K., Bailey T. J., Brockman F., Kieft T. L., Peacock A., White D. C., Balkwill D., Phelps T. J. and Boone D. R. (1997) The deep gold mines of South Africa: windows in the subsurface biosphere. *SPIE International Society of Optical Engineering*, 344–357.
- Onstott T. C., Mislouack B. J., Lin L.-H., Davidson M. M., Gihring T. M., Moser D. P., Fredrickson J. K., Brockman F. J., Pfiffner S. M., Phelps T. J., Pratt L. M., Boice E. A., Sherwood Lollar B., Ward J. A., Lippmann J., Lithaur D. and van Heerden E. (2004) Does the lack of energy limit deep subsurface life? Proceedings of the 104th General Meeting of the *American Society for Microbiology*. New Orleans. May 2004.
- Oremland R. S. and Des Marais D. J. (1983) Distribution, abundance and carbon isotopic composition of gaseous hydrocarbons in Big Soda Lake, Nevada: An alkaline, meromictic lake. *Geochim. Cosmochim. Acta* **47**, 2107–2114.
- Pfiffner S. M., Peacock A. D., White D. C., Phelps T. J., Takai K., Fredrickson J. K., Moser D. P. and Onstott T. C. (2000) Relating subsurface microbial communities to geochemical parameters in samples from deep South African gold mines. *Eos Transactions. American Geophysical Union*, 81(48), Fall Meeting Supplement, Abstract B71A-06.
- Potter J. and Konnerup-Madsen J. (2003) A review of the occurrence and origin of abiogenic hydrocarbons in igneous rocks. In *Hydrocarbons in Crystalline Rocks*, Vol. 214 (ed. N. Petford and K. J. W. McCaffrey), pp. 151–173, Geological Society London.
- Potter J., Rankin A. H., Treloar P. J., Nivin V. A., Ting W., and Ni P. (1998) A preliminary study of methane inclusion in alkaline igneous rocks of the Kola igneous province, Russia: implication for the origin of methane in igneous rocks. *Eur. J. Mineral.* **10**, 1167–1180.
- Raskin L., Stromley J. M., Rittmann B. E., and Stahl D. A. (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Applied and Environmental Microbiology* **60**, 1232–1240.
- Robb L. J. and Meyer F. M. (1995) The Witwatersrand Basin, South Africa: Geological framework and mineralization processes. *Ore Geol. Reviews* **10**, 67–94.
- Rouviere P., Mandelco L., Winker S., and Woese C. R. (1992) A detailed phylogeny for the *Methanomicrobiales*. *Applied Microbiology* **15**, 363–371.
- Schoell M. (1980) The hydrogen and carbon isotopic composition of methane from natural gases of various origins. *Geochimica Cosmochimica Acta* **44**, 649–661.
- Schoell M. (1983) Genetic characterization of natural gases. *American Association of Petroleum Geologists Bulletin* **67**, 2225–2238.
- Schoell M. (1984) Stable isotopes in petroleum research. In *Advances in Petroleum Geochemistry, Vol. 1* (eds. J. Brooks and D. Welte), pp. 215–245. Academic Press, London.
- Schoell M. (1988) Multiple origins of methane in the earth. *Chem. Geol.* **71**, 1–10.
- Schoell M. and Fellmer F.-W. (1981) Anomalous ¹³C depletion in early Precambrian graphites from Superior Province, Canada. *Nature* **290**, 696–699.
- Sherwood Lollar B., Frappe S. K., Fritz P., Macko S. A., Welhan J. A., Blomqvist R., and Lahermo P. W. (1993) Evidence for bacterially generated hydrocarbon gas in Canadian Shield and Fennoscandian Shield rocks. *Geochim. Cosmochim. Acta* **57a**, 5073–5085.
- Sherwood Lollar B., Frappe S. K., Weise S. M., Fritz P., Macko S. A., and Welhan J. A. (1993) Abiogenic methanogenesis in crystalline rocks. *Geochim. Cosmochim. Acta* **57b**, 5087–5097.
- Sherwood Lollar B., Weise S. M., Frappe S. K., and Barker J. F. (1994) Isotopic constraints on the migration of hydrocarbon and helium gases of southwestern Ontario. *Bulletin of Canadian Petroleum Geol.* **42**, 283–295.
- Sherwood Lollar B., Ward J. A., Slater G. F., Lacrampe-Couloume G., J. A. H., Lin L.-H., Moser D. P. and Onstott T. C. (2002a) Hydrogen and hydrocarbon gases in crystalline rocks: Implications for the deep biosphere. *12th Annual V.M. Goldschmidt Conference, Geochimica Cosmochimica Acta Vol. 66, No. 15A*, pp. 706.
- Sherwood Lollar B., Westgate T. D., Ward J. A., Slater G. F., and Lacrampe-Couloume G. (2002) Abiogenic formation of gaseous alkanes in the Earth's crust as a minor source of global hydrocarbon reservoirs. *Nature* **416b**, 522–524.
- Spangenberg J. E. and Frimmel H. E. (2001) Basin-internal derivation of hydrocarbons in the Witwatersrand Basin, South Africa: evidence from bulk and molecular $\delta^{13}\text{C}$ data. *Chem. Geol.* **173**, 339–355.
- Strunk, O. and Ludwig, W. (1995) ARB – a software environment for sequence data (online). Department of Microbiology, Technical University of Munich, Munich, Germany. <http://www.mikro.biologie.tu-muenchen.de/pub/ARB/documentation/arb.ps>.
- Takai K., Moser D. P., DeFlaun M., Onstott T. C., and Fredrickson J. K. (2001) Archaeal diversity in waters from deep South African gold mines. *Applied and Environmental Microbiology* **67**, 5750–5760.
- Tweddie E. B. (1986) The Evander Goldfield. *Mineral Deposits of Southern Africa*. (eds. C. R. Anhauser and S. Maske). Geological Society of South Africa, Johannesburg, South Africa, pp 705–730.
- van Heerden A. W. C. (1998) A lithological and geomorphological study of the VCR sand facies observed within the 4-shaft complex, Kloof Gold Mine. BSc. Hons., University of Stellenbosch.
- Vanko D. A. and Stakes D. S. (1991) Fluids in oceanic layer 3: evidence from veined rocks, hole 735B, Southwest Indian Ridge. *Proceedings of the Oceanic Drilling Program, Scientific Results* **118**, 181–215.
- Voytov G. I. (1991) Chemical and carbon-isotope fluctuations in free gases (gas jets) in the Khibiny. *Geokhimiya* **6**, 769–780.
- Ward J. A. (2002) Identifying Subsurface Gas Origins. Within the Witwatersrand Basin, South Africa. Masters of Science Thesis, University of Toronto.
- Welhan J. A. (1988) Origins of methane in hydrothermal systems. *Chem. Geol.* **71**, 183–198.
- Welhan J. A. and Craig H. (1979) Methane and hydrogen in East Pacific Rise hydrothermal fluids. *Geological Research Letters* **6**, 829–831.
- Whiticar M. J. (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem. Geol.* **161**, 291–314.
- Whiticar M. J., Faber E., and Schoell M. (1986) Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs acetate fermentation—Isotope evidence. *Geochim. Cosmochim. Acta* **50**, 693–709.
- Whittman W. B., Bowen T. L. and Boone D. R. (1999) The methanogenic bacteria. In *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community* (ed. M. Dworkin), Springer-Verlag.
- Woese C. R. and Olsen G. J. (1986) Archaeobacterial phylogeny: perspectives on the urkingdoms. *Syst. Appl. Microbiol.* **7**, 161–177.
- Yuen G. U., Pecore J. A., Kerridge J. F., Pinnavaia T. J., Rightor E. G., Flores J., Wedeking K., Mariner R., Des Marais D. J. and Chang S. (1990) Carbon isotopic fractionation in Fischer-Tropsch type reactions. *Lunar and Planetary Science Conference XXI*, 1367–1368.